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Art. XII.—*Note on the Oil of Subterranean Clover Seed (Midseason Variety).*

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Subterranean Clover forms large quantities of seed which is known to contain a good deal of oil. The following examination of this oil was carried out at the suggestion of Mr. H. A. Mullett, Director of Agriculture for Victoria. The seed was obtained through the Department of Agriculture.

The oil was extracted with ether in a Soxhlet Extractor from the finely ground seed, and, after removal of the ether, it was dried by heating in a boiling water bath, CO_2 being passed through the flask to prevent oxidation. The amount of oil obtained was found to be 16·8 per cent. of the whole seed.

Other samples of oil were obtained by pressure, but it was found necessary to moisten the seed with water (about 5 per cent. of the weight of the seed) for 24 hours before any quantity of oil could be obtained in this way. The finely-ground seed was then pressed in an experimental hydraulic press at a pressure of 1,000-1,500 lb. per square inch. (We are indebted to Mr. H. E. West of the William Angliss Food Trades School for preparing the samples of pressed oil.) The yield of oil by this method was 5-6 per cent.

The chemical tests carried out were the Saponification Value, Reichert-Meissl and Polenske numbers and the Iodine Value. The Saponification Value is a measure of the soluble, volatile fatty acids, i.e., the lower acids up to caproic (C_6), and the Polenske value is a measure of the insoluble volatile fatty acids present (acids from C_6 to about C_{10}). The standard methods were used for these determinations. The Iodine Number, which is an index of the unsaturated acids present, was estimated by Wij's solution (iodine monochloride in glacial acetic).

	Pressed.	*Extracted.*
Saponification Value	201	211
Reichert-Meissl No.	1·6	1
Polenske No.	0·7	0·8
Iodine Value	108	111
Refractive Index at 19·1° C.	1·4680	1·4703
Unsaponifiable Matter	1·97%	—

The results are on the whole slightly higher for the extracted oil. This is probably due to a trace of water in the pressed oil.

A further estimation was made of the unsaturated fatty acids in the oil by the method of Hilditch, which depends essentially

on the differing solubilities of the lead salts of the saturated and unsaturated acids in ether or alcohol. The mixed fatty acids were first prepared. About 20 gm. of oil was saponified with alcoholic potash, and most of the alcohol then removed. The soaps were dissolved in water and the unsaponifiable matter was then extracted with ether. The soaps were next converted into free fatty acids by warming with dilute H_2SO_4 (an atmosphere of nitrogen being used to prevent oxidation) and these acids were then extracted with ether and dried under low pressure at 80–90°C.

To a boiling alcoholic solution of the mixed fatty acids lead acetate in boiling alcohol was added. The solution was then cooled slowly to 15 degrees and left to stand overnight. The insoluble lead compounds were filtered off, the alcohol removed by distillation, and the unsaturated acids remaining were dissolved in ether. This solution was washed twice with dilute acetic acid to set free the acids, and the ethereal solution of these washed with water until the aqueous layer was no longer acid. Any fatty acids retained in the water used in this process were recovered by extracting with a further quantity of ether which was then added to the original ethereal extracts. The last traces of ether and water were removed by heating in a water bath under reduced pressure. The percentage of unsaturated acids was found to be 61·5 per cent.

Summary.

The oil obtained from clover seed contains about 60 per cent. of unsaturated acids, and as the Reichert-Meissl and Polenske values indicate, very few acids either saturated or unsaturated below C_{10}. Its Iodine Value is considerably below that of Linseed and the other oils used for paint manufacture, but since drying properties of oils are not wholly dependent on the Iodine Value, this point may be worth further investigation.

The following table shows that this oil is similar in character to Olive, Maize, and Cotton Seed Oil.

	Saponification Value.	*Iodine Value.*	*Unsaturated Acids.*
Olive Oil	190·5	85	85%
Maize Oil	189·93	115·25	90%
Cotton Seed Oil	191·5	108·16	75%
Clover Seed Oil	201·11	108·11	61%

Tests for the presence of vitamin A were done on the unsaponifiable matter of the oil by the Carr-Price Test (antimony trichloride in chloroform). These tests were entirely negative.

This work has been carried out in the Department of Biochemistry of the University of Melbourne, and thanks are due to Professor W. J. Young for suggesting the line of investigation and for his help and advice throughout.